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## Mapping quantitative trait loci controlling early growth in a (longleaf pine × slash pine) × slash pine BC<sub>1</sub> family

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**Abstract** Random amplified polymorphic DNA (RAPD) markers were employed to map the genome and quantitative trait loci controlling the early growth of a pine hybrid F<sub>1</sub> tree (*Pinus palustris* Mill. × *P. elliottii* Engl.) and a recurrent slash pine tree (*P. elliottii* Engl.) in a (longleaf pine × slash pine) × slash pine BC<sub>1</sub> family consisting of 258 progeny. Of the 150 hybrid F<sub>1</sub> parent-specific RAPD markers, 133 were mapped into 17 linkage groups covering a genetic distance of 1,338.2 cM. Of the 116 slash pine parent-specific RAPD markers, 83 were mapped into 19 linkage groups covering a genetic distance of 994.6 cM. A total of 11 different marker intervals were found to be significantly associated with 13 of the 20 traits on height and diameter growth using MAPMAKER/QTL. Nine of the eleven marker intervals were unique to the hybrid parent 488 genome, and two were unique to the recurrent parent 18–27 genome. The amount of phenotypic variance explained by the putative QTLs ranged from 3.6% to 11.0%. Different QTLs were detected at different ages. Two marker intervals from the hybrid parent 488 were found to have QTL by environment interactions.

**Keywords** *Pinus palustris* · *Pinus elliottii* · QTLs · Random amplified polymorphic DNAs · Early height growth

### Introduction

Longleaf pine (*Pinus palustris* Mill.) has many desirable characters, such as good wood quality, fusiform rust resistance and southern pine bark beetle resistance. However, it has a delay in early height growth (EHG) known as the “grass stage.” The grass stage has been an important factor limiting the artificial regeneration of longleaf pine (Schmidtling and White 1989). Efforts to genetically improve the EHG of longleaf pine through the introgression of genes controlling EHG from either loblolly pine or slash pine began in the 1960s. Brown (1964) and Derr (1966, 1969) made crosses between longleaf pine and loblolly pine or slash pine. C.D. Nelson (unpublished data) made crosses between longleaf pine and slash pine in 1990 and backcrosses in 1995. No “grass stage” was observed in progeny from both crosses, and great variation of height growth was observed among the progeny. Previous studies also revealed that EHG is a quantitative trait controlled by a small number of major effect genes (Brown 1964, Nelson unpublished data) and has a heritability ranging from 0.47 to 0.68 (Snyder and Namkoong 1978; Layton and Goddard 1982). On the basis of these studies, a more efficient approach to genetically improve EHG may be to map these gene loci with molecular markers and then use the markers that are tightly linked to these loci for marker-assisted selection.

While mapping quantitative trait loci (QTLs) controlling the EHG in a (longleaf pine × slash pine) × longleaf pine BC<sub>1</sub> family is the more direct way to address the issue of introgression of EHG genes into longleaf pine, mapping them in a (longleaf pine × slash pine) × slash pine BC<sub>1</sub> family may be more efficient for identifying QTLs originating from the longleaf pine grandparent. We expected to find that the EHG trait of BC<sub>1</sub> individuals is regulated by positive-effect QTLs mainly from slash pine and/or negative-effect genes mainly from longleaf pine and that the homology level between longleaf pine and slash pine is lower than within slash pine species. The negative-effect genes in the F<sub>1</sub> parent that come from the longleaf pine grandparent are less likely to be

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shared by the slash pine parent. Consequently, we expected more genes would be detected in the  $F_1$  parent than in the recurrent parent and that these identified genes would be more likely to be from the longleaf pine grandparent than from the slash pine grandparent. In contrast, if a (longleaf pine  $\times$  slash pine)  $\times$  longleaf pine  $BC_1$  family is used, the genes identified in the  $F_1$  would be more likely to be from the slash pine grandparent. The negative-effect genes identified in a (longleaf pine  $\times$  slash pine)  $\times$  slash pine  $BC_1$  family would provide some extra information that cannot be obtained using a (longleaf pine  $\times$  slash pine)  $\times$  longleaf pine  $BC_1$  family. With this information, we may be able to avoid the EHG QTLs with negative effect while introgressing the ones with positive effect by means of marker-assisted selection in breeding longleaf pine with improved EHG.

Random amplified polymorphic DNAs (RAPDs) markers are attractive markers for genome mapping, QTL mapping, map-based cloning, and analysis of genetic variation for several reasons. RAPDs are polymerase chain reaction (PCR)-based DNA markers. PCR-based markers can be used to map repetitive regions of a genome as efficiently as they can map the gene-rich regions (Monna et al. 1994). This property is one of the advantages that PCR-based markers have over cDNA-based restriction fragment length polymorphism (RFLP) markers (Gill et al. 1996a, b). This is particularly significant in mapping genomes of organisms that have large genomes where repetitive DNAs comprise a large proportion of the genome (Miksche and Hotta 1973; Rake et al. 1980; Kriebel 1985). The genome size of *Pinus* species has been estimated to be between 33 and 57 pg (equivalent to about  $3 \times 10^{10}$  bp per/haploid genome) (Ohri and Khoshoo 1986; Walkamiya et al. 1993; Plomion et al. 1995), which is relatively large compared to most other organisms. Besides the properties that are shared with other PCR-based DNA markers, RAPD analysis is fast and simple and uses trace amounts of DNA template (Welsch and McClelland 1990; Williams et al. 1990). RAPDs have been the most frequently used molecular markers for mapping pines, including maritime pine (Plomion et al. 1995), sugar pine (Devey et al. 1995), longleaf pine (Nelson et al. 1993; Kubisiak et al. 1995), slash pine (Nelson et al. 1994; Kubisiak et al. 1995) and radiata pine (Emebiri et al. 1998).

In this paper, we present the RAPD genetic linkage maps of a longleaf pine  $\times$  slash pine  $F_1$  tree and slash pine tree 18–27 using a  $BC_1$  mapping population and detect QTLs using a single marker-based SAS procedure, analysis of variance (ANOVA), and the marker interval-based computer program, MAPMAKER/QTL.

## Materials and methods

### Mapping population

Progeny from a (longleaf pine  $\times$  slash pine)  $\times$  slash pine  $BC_1$  family were used for genome mapping and QTL mapping. The hybrid parent (tree 488,  $F_1$ , ♀) was developed by Derr (1966) by crossing

a longleaf pine (tree 8R, ♀) with a slash pine (tree 51, ♂), and the recurrent parent 18–27 (♂) is a superior selection originally chosen for growth rate, form, and disease tolerance. Seeds of this backcross were germinated in June of 1996 and grown in containers (10 cubic inches of rooting volume) in a greenhouse. During this period all seedlings were protected from diseases (primarily damping-off and brown spot needle blight) and given uniform growing conditions. In January of 1997, the seedlings were planted at three different field locations [southwest Georgia (GA; 92 seedlings), east-central Louisiana (LA; 92 seedlings) and southeast Mississippi (MS; 101 seedlings)] using a completely randomized design with a between-tree spacing of 10 feet  $\times$  10 feet. The overall survival rate was 90.5%, leaving a total of 258 seedlings for linkage mapping and QTL searching (82 at GA site, 83 at LA site and 93 at MS site).

### Field data

As of November 1999, a total of eight measurements had been taken on the  $BC_1$  family. These measurements included total height (h1, h2, h3 and h4) from ground level to bud tip and stem diameter (d1, d2, d3 and d4) at 3–5 cm above the ground on the 7th (in the greenhouse), 16th, 29th, and 41st month (in the field). Measurements were made in January 1997 (prior to out-planting from the greenhouse), October 1997, November 1998, and November 1999. Changes in height and diameter between these measurement dates were also calculated. In total, there were 20 traits: height at the 7th month (h1), 16th month (h2), 29th month (h3), 41st month (h4); height increments from the 7th to the 16th month ( $\Delta h12$ ), 7th to the 29th month ( $\Delta h13$ ), 7th to the 41st month ( $\Delta h14$ ), 16th to the 29th month ( $\Delta h23$ ), 16th to the 41st month ( $\Delta h24$ ), 29th to the 41st month ( $\Delta h34$ ); collar diameter at the 7th month (d1), 16th month (d2), 29th month (d3), 41st month (d4); diameter increments from the 7th to the 16th month ( $\Delta d12$ ), 7th to the 29th month ( $\Delta d13$ ), 7th to the 41st month ( $\Delta d14$ ), 16th to the 29th month ( $\Delta d23$ ), 16th to the 41st month ( $\Delta d24$ ), 29th to the 41st month ( $\Delta d34$ ). For the marker-QTL analysis all traits except h1 and d1 were standardized for location effects as follows:  $Y_{\text{hat}_{ij}} = (Y_{ij} - Y_i) / S_{Y_i}$ , where  $Y_{ij}$  is the actual trait measurement taken on the  $j^{\text{th}}$  individual planted at site  $i$ ;  $Y_i$  is the trait mean for site  $i$ ;  $S_{Y_i}$  is the standard deviation of the trait for planting site  $i$ . Each of the 20 traits was tested for normality using the Wilk-Shapiro test. These tests suggested that four of the traits (d2,  $\Delta d24$ ,  $\Delta d34$  and  $\Delta h34$ ) were not normally distributed. As transformations using log, square root, square and inverse could not obtain normality for any of the measures, these measures were analysed based on a non-normal distribution.

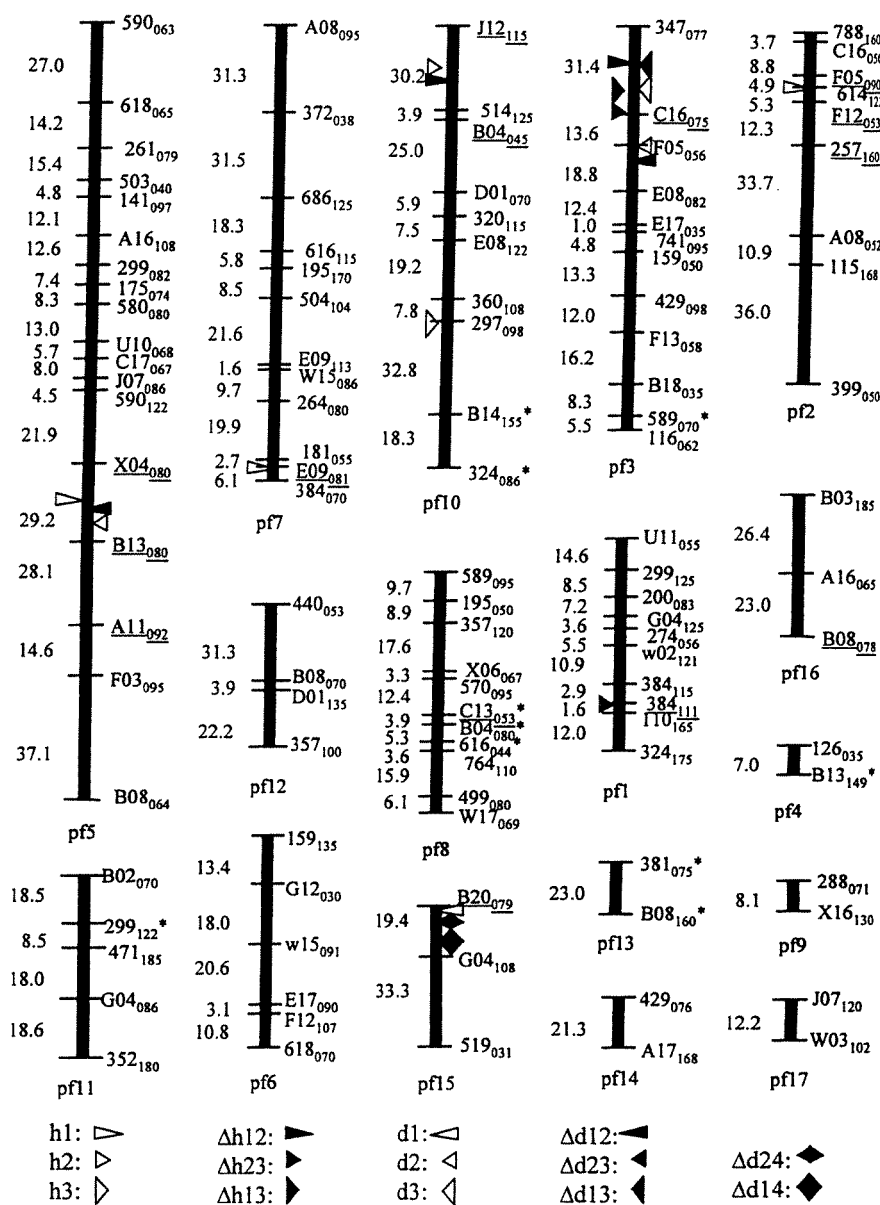
### DNA isolation and purification

Total DNA was extracted from 1.0 g leaf samples of individual trees using a CTAB procedure (Murray and Thompson 1980). The DNA samples were purified further using Prep-A-Gene (Bio-Rad, Richmond, Calif.). The DNA samples were diluted to a working concentration of approximately 20 ng/ $\mu$ l with low TE (10 mM Tris:0.1 mM EDTA).

### RAPD analysis

Decamer primers were purchased from either Operon Technologies (Alameda, Calif.) or J. Hobbs (University of British Columbia, Vancouver, B.C., Canada). Primers were selected either randomly or because they had previously identified polymorphic RAPD markers in longleaf pine or slash pine. To identify useful polymorphisms, we screened primers against the  $F_1$  parent, recurrent parent and six  $BC_1$  progeny. Primers that amplified testcross loci (segregating in 1:1) were further characterised on the entire mapping population ( $n=258$ ). Presence of a band was scored as an 'H' (heterozygous), while absence of a band was scored as 'A' (homozygous band absent). Those cases in which a reaction

**Fig. 1** Linkage maps for the hybrid parent 488. The last digit of all markers was truncated. Markers with an asterisk (\*) were distorted from a 1:1 segregation ratio ( $P < 0.01$ ). Underlined markers were associated with QTLs detected using ANOVA. Loci marked with arrows represent the most likely locations of QTLs using MAPMAKER/QTL



completely failed or when the presence or absence of bands was unclear, were recorded as missing data. RAPDs were named by the manufacturer primer code corresponding to the primer responsible for their amplification, followed by a three-digit number indicating the approximate fragment size in base pairs, with the last digit being the tens position digit of fragment size in base pairs (the ones position digit was truncated).

#### Linkage analysis

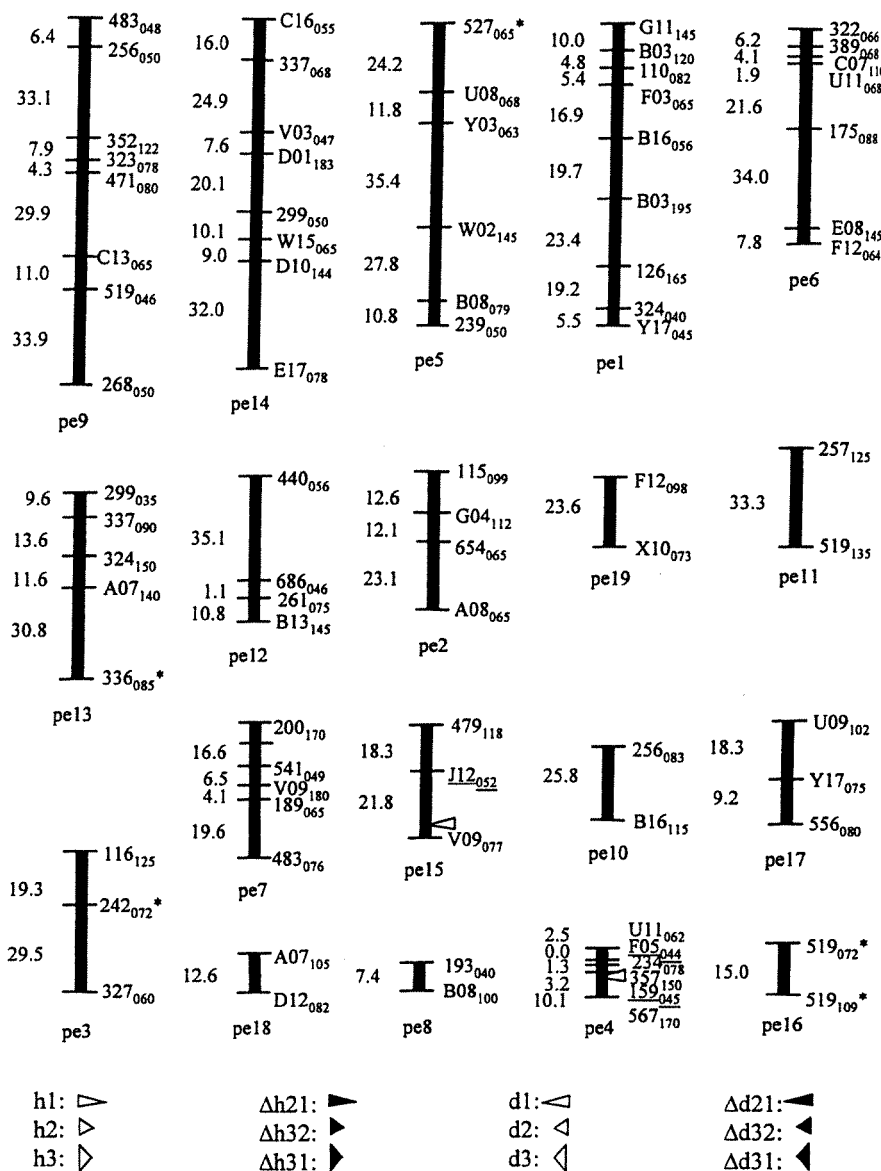
The RAPD data were divided into two subsets; one consisting of all the markers heterozygous in the hybrid parent 488, and a second consisting of all the markers heterozygous in the recurrent parent 18-27; each subset was analysed separately. The marker data were entered into the computer package MAPMAKER/EXP 3.0 (Lander et al. 1987) and analysed using a modified backcross format (Nelson et al. 1993). Each marker was tested for goodness of fit to its expected Mendelian inheritance ratio using chi-square ( $\chi^2$ ) analysis ( $P < 0.01$ ). Linkage groups were established using a minimum log of odds (LOD) threshold of 4.0 and maximum dis-

tance of 40 cM. Those loci that appeared to be experiencing segregation distortion were first excluded from linkage analyses and were placed in their most likely positions after the non-distorted markers were ordered. Linkage groups were assigned a two-letter name followed by a number. The letter designation 'pf' indicates a hybrid parent-specific linkage group, and 'pe' indicates a recurrent parent-specific linkage group.

#### QTL analysis

Two different methods were employed to investigate the degree of association between the marker loci and each of the 20 traits. The first method was single marker-based SAS ANOVA in which the individual marker genotypes were used as class variables. An association between a marker and trait was considered significant if the Type-I error rate was less than 0.005. The interaction between marker and planting site was considered significant if the Type-I error rate was less than 0.005. The proportion of the phenotypic variance explained by segregation of the marker was determined by the R-square ( $R^2$ ) value. The second method utilised the

**Fig. 2** Linkage maps for the slash pine parent 18–27. The last digit of all markers was truncated. Markers with an asterisk (\*) were distorted from 1:1 segregation ratio ( $P < 0.01$ ). Underlined markers were associated with QTLs detected using ANOVA. Loci marked with arrows represent the most likely locations of QTLs using MAPMAKER/QTL



interval mapping approach available in the software package MAPMAKER/QTL 1.0 (Lander et al. 1987). Although there are no statistical inferences available to directly test for marker interval by planting site interaction in MAPMAKER/QTL 1.0, the data for each planting site were analysed separately and the difference in LOD scores between sites used as an indicator of interaction. An association between a marker interval and trait was considered significant if the LOD score observed was greater than 2.0. The interaction between a marker interval and planting site was considered significant if the LOD score difference between any two sites was greater than 2.0.

## Results

### Linkage mapping

A total of 266 RAPD markers (150 heterozygous in the hybrid parent 488 and 116 heterozygous in recurrent parent 18–27) were scored on the BC<sub>1</sub> population. Chisquare

tests suggested that 215 (80.8%) of these markers (119 heterozygous in the hybrid parent 488 and 96 heterozygous in recurrent parent 18–27) segregate at a ratio of 1:1, while the remaining 51 (19.2%) (31 heterozygous in the hybrid parent 488 and 20 heterozygous in recurrent parent 18–27) are distorted from the 1:1 ratio.

Based on two-point analyses, 113 of the 150 hybrid parent 488-specific marker loci were grouped into 17 groups (5 with two loci, 12 with three or more loci). These markers covered a genetic distance of 1,338.2 cM (Fig. 1). Genome size in pine has been estimated to be approximately 2,300–2,400 cM (Plomion et al. 1995; Echt and Nelson 1997) using the method described by Hulbert et al. (1988). Assuming that each of the 23 unlinked markers accounts for 20 cM and that each of the 34 ends of our 17 groups cover 10 cM, the total map coverage is estimated at 2,138.2 cM or 91.0% of the pine genome.

**Table 1** RAPD markers significantly associated with the inheritance of various growth measurements in a (longleaf pine × slash pine) × slash pine BC<sub>1</sub> family based on single-marker ANOVA models ( $P < 0.005$ ). (LG linkage group · UL unlinked marker)

Marker <sup>a</sup>	Parent <sup>b</sup>	LG	Trait <sup>c</sup>	df	F value	Pr>F <sup>d</sup>	R <sup>2e</sup>	Δdf
159 <sub>045</sub>	18_27	pe4	d1	252	10.36	0.0015	0.0403	-0.4056
257 <sub>160</sub>	488	pf2	h1	250	9.11	0.0028	0.0352	0.3818
384 <sub>080</sub>	488	UL	h3	252	8.45	0.0040	0.0328	0.3662
384 <sub>111</sub>	488	pf1	Δh23	239	8.28	0.0044	0.0340	-0.3721
503 <sub>037</sub>	18_27	UL	d2	158	9.12	0.0030	0.0552	0.4805
618 <sub>060</sub>	18_27	UL	d1	83	12.18	0.0008	0.1423	0.7661
A11 <sub>092</sub>	488	pf5	d2	160	10.40	0.0015	0.0618	-0.5100
B04 <sub>045</sub>	488	pf10	h2	234	11.39	0.0009	0.0472	-0.4411
			Δh12	234	10.04	0.0017	0.0418	-0.4143
B08 <sub>078</sub>	488	pf16	d4	166	8.40	0.0043	0.0482	0.4498
			Δd14	166	8.46	0.0041	0.0485	0.4516
			Δd24	154	8.73	0.0036	0.0537	0.4765
B13 <sub>080</sub>	488	pf5	d2	158	11.68	0.0008	0.0697	0.5438
			Δd12	157	10.15	0.0017	0.0561	0.5086
B20 <sub>079</sub>	488	pf15	d1	250	8.78	0.0033	0.0386	-0.3749
			Δd14	244	8.83	0.0033	0.0349	-0.3805
			Δd24	152	9.99	0.0019	0.0617	-0.5127
			Δd34	244	8.27	0.0044	0.0328	-0.3682
C13 <sub>053</sub>	488	pf8	d4	246	8.20	0.0046	0.0322	0.3652
C16 <sub>075</sub>	488	pf3	h3	251	9.91	0.0018	0.0384	0.3974
			Δh13	251	8.98	0.0030	0.0352	0.3782
			Δh23	238	8.71	0.0035	0.0359	0.3826
			d3	251	9.89	0.0019	0.0386	-0.3970
			Δd13	251	9.32	0.0025	0.0365	-0.3855
E09 <sub>081</sub>	488	pf7	h1	250	16.54	0.0001	0.0620	0.5144
F05 <sub>044</sub>	18_27	pe4	d1	253	9.04	0.0029	0.0333	-0.3781
F05 <sub>090</sub>	488	pf2	h1	251	12.30	0.0005	0.0467	-0.4428
F12 <sub>053</sub>	488	pf2	h1	252	10.86	0.0011	0.0413	0.4152
G04 <sub>108</sub>	488	pf15	Δd34	239	8.02	0.0050	0.0325	-0.3664
J12 <sub>052</sub>	18_27	pe15	d1	253	8.32	0.0043	0.0297	-0.3626
J12 <sub>115</sub>	488	pf10	h2	237	14.22	0.0002	0.0568	0.4897
			Δh12	240	10.08	0.0017	0.0410	0.4098
			Δd14	247	8.14	0.0047	0.0319	0.3630
W03 <sub>102</sub>	488	pf17	Δd34	80	9.17	0.0033	0.1028	-0.6773
X04 <sub>050</sub>	488	UL	Δh14	80	8.74	0.0041	0.0985	-0.6611
			Δh34	80	10.80	0.0015	0.1189	-0.7348
X04 <sub>080</sub>	488	pf5	h1	251	10.85	0.0011	0.0415	0.4158

<sup>a</sup> Marker locus

<sup>b</sup> Informative parent

<sup>c</sup> Growth metric

<sup>d</sup> Probability of a larger F value

<sup>e</sup> R-square or the proportion of the phenotypic data explained by the marker locus

<sup>f</sup> Difference between the QTL allele and the population mean expressed in phenotypic standard deviations

Of the 116 recurrent parent 18–27-specific markers 87 were grouped into 19 groups, covering a total genetic distance of 994.6 cM (Fig. 2). Adjusting for the 29 unlinked loci and the 38 ends of the 19 linkage groups, the total map coverage is estimated at 1,954.6 cM or 81.1% of the pine genome.

#### Detecting QTLs using the single marker method

A total of 23 different markers were found to be significantly associated with QTLs for 17 of the 20 growth traits using single marker regression (Table 1). No markers were found to be significantly associated with diameter increments from the 16th to the 29th month (Δd23) and from 41th to 16th month (Δd24), and total height at 41th month (h4). Of the 23 significant associations, 18 were unique to the hybrid parent 488 genome, and five were unique to the recurrent parent 18–27 genome. Of the 18 hybrid parent 488-specific markers, nine were associated only with height growth, seven were associated only with diameter growth, with two being associated with both height and diameter growth. Two markers were significantly associated with growth metrics at more than

one age (Table 1). Of these 18 markers, 16 were located on ten different hybrid parent 488-specific linkage groups (Fig. 1) and two were unlinked with any other markers. The amount of variation explained by the various QTLs ranged from 3.19% (0.3630σ) to 11.89% (0.7348 σ) of the total phenotypic variance. All five markers unique to recurrent parent 18–27 were associated with diameter growth measurements. Three of these were located on two different linkage groups (Fig. 2), while two markers were unlinked. The amount of variation explained by these five QTLs ranged from 2.97% (0.3626σ) to 14.23% (0.7661σ) of the total phenotypic variance.

#### Detecting QTLs using MAPMAKER/QTL

A total of 11 different marker intervals were found to be significantly associated with 13 of the 20 growth traits using the interval mapping method of MAPMAKER/QTL (Table 2). Nine of the eleven marker intervals were unique to the hybrid parent 488 genome, and two were unique to the recurrent parent 18–27 genome. Of the nine hybrid parent 488 marker intervals, five were

**Table 2** RAPD marker intervals significantly associated with the inheritance of various growth measurements in a (longleaf pine × slash pine) × slash pine BC<sub>1</sub> family based on the interval mapping method (LOD>2.0) (LG linkage group)

Interval <sup>a</sup>	Parent <sup>b</sup>	LG	Trait	LOD	Variance explained <sup>c</sup> (%)	Substitution effect
159 <sub>045</sub> –567 <sub>170</sub>	18_27	pe4	d1	2.37	4.1	Positive
297 <sub>098</sub> –B14 <sub>155</sub>	488	pf10	h3	2.01	3.6	Negative
347 <sub>077</sub> –C16 <sub>075</sub>	488	pf3	Δh12	2.05	5.0	Negative
			Δh23	2.18	4.3	Negative
			Δh13	2.02	4.5	Negative
			d3	2.54	6.1	Negative
			Δd13	2.54	6.1	Negative
384 <sub>111</sub> –110 <sub>165</sub>	488	pf1	Δh23	2.76	5.1	Positive
B04 <sub>045</sub> –J12 <sub>115</sub>	488	pf10	Δh12	3.29	8.5	Negative
			h2	4.34	11.0	Negative
B20 <sub>079</sub> –G04 <sub>108</sub>	488	pf15	d1	2.29	4.0	Negative
			Δd14	2.07	4.2	Negative
			Δd24	2.38	7.8	Negative
E09 <sub>081</sub> –264 <sub>080</sub>	488	pf7	h1	3.40	5.9	Negative
F05 <sub>056</sub> –E08 <sub>082</sub>	488	pf3	d2	2.20	6.3	Negative
			Δd12	2.40	6.9	Negative
F05 <sub>090</sub> –F12 <sub>053</sub>	488	pf2	h1	2.80	5.4	Positive
J12 <sub>052</sub> –V09 <sub>077</sub>	18_27	pe15	d1	2.01	5.7	Negative
X04 <sub>080</sub> –B13 <sub>080</sub>	488	pf5	h1	2.35	4.8	Positive
			d2	2.71	10.0	Negative
			Δd12	2.74	11.0	Negative

<sup>a</sup> Marker interval

<sup>b</sup> Informative parent

<sup>c</sup> Percentage of the phenotypic data explained by the marker interval

**Table 3** Markers that were associated with QTL by environmental interaction using simple regression and a Type-I error rate of 0.005. See Table 1 for definitions

Marker	Parent	LG	Trait	F value	Pr>F	R <sup>2</sup>
590 <sub>063</sub> *site	488	pf5	d2	10.67	0.0013	0.0640
618 <sub>065</sub> *site	488	pf5	d2	8.15	0.0049	0.0497
590 <sub>063</sub> *site	488	pf5	Δd12	9.63	0.0023	0.0581

associated only with height growth, two were associated only with diameter growth, and two were associated with both height and diameter growth. Two of the marker intervals were significantly associated with growth metrics at more than one age (Table 2). These nine marker intervals were located on seven different hybrid parent-specific linkage groups (Fig. 1). The amount of variation explained by the various QTLs ranged from 3.60% to 11.0% of the total phenotypic variance. The two marker intervals in the recurrent parent 18–27 genome were only associated with diameter growth at one age (Table 2). These two intervals were located on two different linkage groups (Fig. 2). These QTLs explained 4.1% and 5.7% of the total phenotypic variance, respectively.

#### QTL by environment interactions

Only two QTL by planting site interactions were found to be significant – marker 590<sub>1175</sub> associated with height growth at the 7th month (h1) and marker 347<sub>0780</sub> associated with the change in diameter growth from the 7th to the 19th month. Both markers were from the hybrid parent 488 (Table 3) and were located in linkage group pf5. Two intervals, 347<sub>077</sub>–C16<sub>075</sub> and X04<sub>080</sub>–B13<sub>080</sub>, both

**Table 4** Intervals that were associated with QTLs by environmental interactions

Interval	LG	Trait	LOD			Combined LOD
			GA site	LA site	MS site	
X04 <sub>080</sub> –B13 <sub>080</sub>	pf5	d2	–	0.49	2.57	3.18
347 <sub>077</sub> –C16 <sub>075</sub>	pf3	Δd12	–	2.70	0.24	1.74
X04 <sub>080</sub> –B13 <sub>080</sub>	pf5	Δd12	–	0.47	2.77	3.18

from the hybrid parent 488, showed interaction effects. Interval 347<sub>077</sub>–C16<sub>075</sub> was from linkage group pf3 and interval X04<sub>080</sub>–B13<sub>080</sub> was from linkage group pf5 (Table 4).

#### Discussion

Twenty-three markers were found to be associated with 17 of the 20 traits using ANOVA, with variance explained by associated QTLs ranging from 2.97% (0.3626σ) to 14.23% (0.7661σ) of the total phenotypic variance. Twenty-one QTLs influencing 13 of the 20 traits were located in 11 different marker intervals using MAPMAKER/QTL, with the variance, explained by associated QTLs, ranging from 3.60% to 11.0% of the total phenotypic variance. The results showed a trend: the amount of variance explained jointly by all major-effect QTLs influencing the same measurement became smaller when trees became older. At the 7th month, the amount of variance explained jointly by all QTLs was 14.5% and 12.1% for h1 and d1, respectively, when analysed using multiple regression, while no major QTLs were detected for Δh34 and Δd34. Two markers were found to associate with interactions between three QTLs and their environments using ANOVA. Two intervals showed

QTL by environment interaction using MAPMAKER/QTL. No marker was found to associate with QTLs that have significant effects on all the four ages.

Markers detected to be associated with the traits using MAPMAKER/QTL were similar to those detected using ANOVA, and the two methods could be used complementarily. Of the 19 linked markers associated with QTLs detected using ANOVA, 13 were found in the intervals associated with QTLs detected using MAPMAKER/QTL, and three markers, 257<sub>160</sub>, A11<sub>092</sub> and F05<sub>044</sub>, were found to link (at genetic distance of 12.3, 28.1, and 4.5 cM, respectively) with intervals associated with QTLs detected using MAPMAKER/QTL, which suggested that both methods are feasible for QTL detection. ANOVA and MAPMAKER/QTL could be used complementarily. First, except for the case of marker E09<sub>081</sub> versus interval E09<sub>081</sub>–264<sub>080</sub>, the QTL effects estimated by MAPMAKER/QTL were always greater than those estimated by ANOVA. On average, the percentage variance explained by each interval using MAPMAKER/QTL was 0.021 higher than the highest R-square value partitioned by the corresponding marker in a linkage group using ANOVA. One explanation could be that misclassification of QTL genotypes occurs whenever there is a crossover between the QTL and the marker (Weng et al. 1999) and that this misclassification will result in smaller mean difference and lead to a decrease in R-square. This may imply that MAPMAKER/QTL is more powerful than ANOVA. Second, unlinked markers that cannot be used by MAPMAKER/QTL can be analysed using ANOVA because no marker linkage information is needed for ANOVA to detect marker-QTL association. Four QTLs were found to be associated with four markers unlinked to any other markers. The marker 618<sub>060</sub>, one of these four, explained 14.23% of the total phenotypic variance of trait d1.

In terms of amount of variance explained by the QTLs, the results were comparable to results published previously for some other pine species. The three QTLs associated with h1 in MAPMAKER/QTL analysis explained 14.5% of the total phenotypic variance of h1, and the three QTLs associated with d1 explained 12.1% of the total phenotypic variance of d1. Compared to results from some well-studied plant species, these numbers were low. In soybean, a QTL for plant height was found to explain 67.7% of the total phenotypic variance, a number almost five times as much as the one we obtained for our populations. In the outcrossing species *Eucalyptus nitens*, three QTLs, each with an effect between 10% and 15%, have been detected for the total height of seedlings at 55 days after planting out (Byrne et al. 1997). However, our results were not unusual when we compared them to published results for maritime pine (two QTLs explaining 17% of total phenotypic variance of height at 15 weeks, three QTLs explaining 21% of height at 38 weeks and one QTL explaining 10% of height at 92 weeks) (Plomion et al. 1996), loblolly pine (one QTL explaining 20% of total phenotypic variance of height increment at age 2 years and one QTL explaining 12.6% of height increment at age 4 years) (Kaya et al.

1999) and cocoa (two QTLs explaining 16.5% of total variance of height and two QTLs explaining 11.2% of total variance of diameter of two-year-old cocoa) (Crouzillat et al. 1996).

The information about from which grandparent the QTLs detected in the F<sub>1</sub> parent were derived is not a must for marker-assisted selection. Due to the lack of linkage information for the grandparents, we could not determine the derivation of the QTLs detected in the F<sub>1</sub> parent. Thus, we could not tell whether a band-present-associated negative effect was due to a band-present-associated negative effect allele, which would be expected to be more likely from the longleaf pine grandparent, or a band-absent-associated positive effect allele, which would be expected to be more likely from the slash pine grandparent. However, this situation would not be a problem in marker-assisted selection. When selecting a QTL from the F<sub>1</sub> parent using marker-assisted selection, selecting individuals that contain a band-present-associated positive effect allele or avoiding individuals that contain a band-present-associated negative effect allele would be adequate for selection on this QTL. Of course, with information on the derivation of these QTLs, we would be able to increase the certainty of the existence of these QTLs.

The results from this research may suggest some recommendations for future longleaf pine breeding programs for early height and diameter growth. First, more markers will be needed to cover the genome of the population. Without taking the unlinked markers into account, only about 70% and 58% of the genome was covered. With more markers, more QTLs located in the unmapped-regions may be detected. Second, the interactions across planting sites suggest that different lines should be developed for different planting sites. Developing a set of lines for a series of environments may facilitate the breeding program. Third, more loci will need to be taken into account than we had expected. The number of loci involved in regulating height and diameter growth was estimated to be five. However, our results have shown that different QTLs were activated at different growth ages and, consequently, the total number we need to work on may be more than ten, with about three for each age.

There were some limitations in this experiment. First, the results of this research can only provide some extra information about QTLs, mainly the negative-effect ones from the longleaf pine grandparents. None of the BC<sub>1</sub> individuals were designed to make any further crosses. Second, many putative QTLs had a low LOD score. Those loci were subjected to further tests to confirm their existence of EHG effects. The LOD scores for 12 of the 21 QTLs were smaller than 2.5, and 18 of the 21 QTLs were smaller than 3.0. The reasons for the low LOD scores may be small sample size for each environment, small effects of the corresponding QTLs compared to the environment effects or spurious QTLs detected. By increasing the sample size, LOD scores could be increased, and the probability of detecting spurious QTLs could be decreased.

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